accompanied by generic terminology. Applicant have amended the specification as per the Examiner's suggestion. Thus no new matter is added by these amendments.

Amendments to the Claims

Claims 1-20 are pending in this application. Support for the amendments to the claims may be found throughout the specification. For example, support for the amendment to claim 1, 2, 10 and 16 may be found at page 4, lines 13-16, page 5, lines 15-19, page 19, lines 21-30, page 20, lines 1-30, page 21, lines 1-30 and Example 1. Thus, no new matter is added by these amendments.

The amendments to claims 3, 7, and 12 are being made to comply with the requirements for Sequence Listing Rules under 37 C.F. R. § 1.821-§ 1.825. The amendments assign sequence identifier numbers to sequences presented in the claims as required by 37 C.F.R. 1.821(d). Thus, no new matter is added by these amendments. Reconsideration of the application in view of the following remarks is respectfully requested.

OBJECTIONS TO INFORMALITIES IN THE SPECIFICATION

The disclosure has been objected to as containing informalities because the specification does not comply with the requirements of 37 C.F.R §1.821-§1.825. Specifically, the Examiner contends that the sequences presented in the claims lack sequence identifiers. Applicant has amended the claims so each sequence is now identified by a sequence identification number. In addition the specification was objected to because the Examiner contends that the use of the trademark Taqman™ was not capitalized when recited or accompanied by generic terminology. Applicant have amended the specification as per the Examiner' suggestion (e.g., real time PCR (TAQMAN™ PCR)). Accordingly, Applicant respectfully requests withdrawal of this objection.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claim 16 is rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. Specifically, the Examiner contends that the claiming of a "kit" appears to represent new matter. Applicant respectfully traverses this rejection for the reasons presented below.

A term recited in a claim need not be described literally in order for the specification to satisfy the description requirement [In re Lukach 442 F.2d 967, 969; 169 USPQ 795 (CCPA 1971)]. All that is necessary is that the specification "convey to those skilled in the art, to whom it is addressed , in any way, the information that applicant had invented the specific subject matter later claimed [In re Wartheim, 541 F2d 257, 262; 191 USPQ 90, 97 (CCPA 1976)]. The instant specification discloses "sets" of primers and probes for the detection and differentiation of pathogenic *E. coli* (see for e.g., page 6, lines 8-11, page 9, lines 1-30 and page 10, lines 1-10, page 21, lines 26-30 etc). One of skill in the art would understand that such "sets" of primers and/or probes are often formulated as a kit. Accordingly, while the recitation "kit" is not literally recited in the specification, one of skill in the art would understand that the invention encompassed such kits. Accordingly, applicant was in possession of the invention as claimed and respectfully request withdrawal of this ground of rejection.

REJECTIONS UNDER 35 U.S.C. § 112, SECOND PARAGRAPH

Claims 2-, 13, 14 and 15 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner contends that claims 2 and 3 are indefinite because "it is not clear if the claim requires that the two primer pairs be selected from the "larger groups" listed or if the two primer pairs may be selected from any of those described, wherein the "larger groups" are the ones designated by a dash at the beginning of the line."

(Office Action, bridging paragraph, pages 4 and 5). The recitation "two or more" has been deleted from claim 2, thereby rendering the Examiner's rejection with respect to claims 2 and 3 moot.

The Examiner also contends that claims 4-9 are indefinite over the recitation of "a fluorescent quencher dye which hybridizes within the target DNA" because it is not clear if the quencher dye is intended to hybridize or if the previously mentioned oligonucleotide probe is intended to hybridize with the target DNA (Office Action, page 5). For clarity, claim 4 has been amended to recite that the "probe hybridizes with the target DNA". Accordingly, as amended claims 4 –9 are believed to satisfy the statute.

The Examiner rejected claims 13, 14, 15, and 16 were rejected by the Examiner because of the use of the trademark/trade name TaqMan™-PCR. As amended, claims 13-15 are believed to comply with the statute. Applicant has replaced the recitation "TaqMan™ PCR" in claim 13 and 16 with the recitation --real time –PCR--. As amended, claims 13-15 are believed to comply with the statute.

REJECTIONS UNDER 35 U.S.C. § 102

Claims 1, 2, 10, 11, 13, and 14 are rejected under 35 U.S.C. 102 as being anticipated by Lang *et al.* (Applied and Environmental Microbiology, Sept. 1994, p. 3145-3149). Applicant traverses this ground rejection with respect to the newly amended claims for the reasons discussed below.

For a finding of anticipation, no difference must exist between the claimed invention and the reference. Specifically, anticipation requires that each and every element as set forth in the claim be present in a single prior art reference. See Davis v. Loesch, 27 U.S.P.Q. 2d 1440 (Fed. Cir. 1993). Applicant's claimed invention is directed to methods (e.g., claims 1, 4, 5, 8, 9 and 17 -20) and sets of primers and probes (e.g., claims 10, 13 and 16) for detection and differentiation of the subgroups of pathogenic *E. coli* which are associated with diarrhea, food-borne illnesses etc. In contrast, Lang *et al.* relates to the differentiation of enterotoxigenic and enterohemorrhagic *E. coli* strains by amplification of a toxin gene characteristic for one of the

two subgroups of *E. coli* using a set of oligonucleotide primer pairs (see Abstract). Exclusion of a single claimed element from the prior art reference is enough to negate anticipation by that reference. See Atlas Powder Co. v. E.I. du Pont De Nemours & Co. 224 U.S.P.Q. 409, 411 (Fed. Cir. 1984). As the Lang et al. reference does not disclose each aspect of the claimed invention, the reference fails to anticipate the claimed invention. Reconsideration and withdrawal of the § 102 rejection is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 103

Claims 3, 12, and 15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* in view of Yamamoto *et al.* (Journal of Bacteriology, Aug. 1983, Vol. 155, No. 2, p. 728-733) and further in view of Hogan *et al.* (US 5595874). Applicant traverses this ground of rejection with respect to the newly amended claims for the reasons discussed below.

As discussed above, Applicant's claimed invention is directed to methods (e.g., claim 3) and sets of primers and probes (e.g., claims 12 and 15) for detection and differentiation of all five subgroups of pathogenic *E. coli* are associated with food born illnesses etc. As noted by the Examiner, Lang *et al.* does not teach or suggest "methods or sets of primers and probes which include instant SEQ ID NO: 1, 2, or 19." (Office Action, page 7). In addition, as discussed above, Lang *et al.* relates to the amplification of a toxin gene characteristic for one of the two subgroups (e.g., enterotoxigenic and enterohemorrhagic *E. coli* strains) and not detection and differentiation of all five subgroups of pathogenic *E. coli.* Yamamoto also relates to the full length sequence of the heat labile toxin gene of enterotoxigenic *E. coli* strains, but does not teach or suggest the specific primers or probes used to detect the heat labile toxin gene of enterotoxigenic *E. coli* strains claimed in the instant invention. In addition, Yamamoto does not teach or suggest primers and probes for detection and differentiation of all five subgroups of pathogenic *E. coli.* Such a disclosure is found only in the instant application. thus neither Lang et al or Yamamoto et al either alone or in combination render the claimed invention obvious.

Hogan *et al.* relates to the use of primers for detection of non-viral organisms and provides general guidance for selection of probes or primers. Hogan et al does not teach or suggest the specific primers or probes claimed in the instant application, nor does Hogan teach or

suggest how to select and design probes capable of detecting and differentiating the five subgroups of pathogenic *E. coli*. As Hogan does not remedy the deficiencies of Lang et al or Yamamoto, Hogan et al either alone or in combination cannot render the claimed invention obvious. Therefore, Applicant respectfully request withdrawal of this ground of rejection.

Claims 4-6, 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* in view of Livak *et al.* (PCR Methods and Applications (1995) 4:357-362). Applicant traverses this ground of rejection with respect to the newly amended claims for the reasons discussed below.

As discussed above Lang *et al.* do not teach or suggest methods for the detection and differentiation of five subgroups of pathogenic *E. coli*. As noted by the Examiner, Lang et al does not teach methods utilizing a polymerase having 5'-3' exonuclease activity is used for the amplification of DNA and a probe labeled at both ends is used to detect amplified samples. Livak *et al.* relates generally to the assay method of using oligonucleotide probes with fluorescent dyes to provide a quenched probe. Livak et al does not teach or suggest the use of the method for the detection of five subgroups of pathogenic *E. coli*, nor does Livak teach the specific probes or primers used in the claimed invention. Accordingly, Livak et al either alone or in combination with Lang cannot render the claimed invention obvious, therefore Applicant respectfully request withdrawal of this ground of rejection.

Claim 7 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* in view of Livak *et al.* as applied to claims 4-6, 8 and 9 above, and further in view of both Yamamoto *et al.* and Hogan *et al.* Applicant traverses this ground of rejection with respect to the newly presented claims for the reasons discussed below.

Claim 7 is directed to a method of detecting five subgroups of pathogenic *E. coli* utilizing a specific set of probes. For the reasons discussed herein and above these references either alone or in combination do not render the claimed invention obvious. Accordingly, Applicant respectfully request withdrawal of this ground of rejection.

Claim 16 is rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* in view of Livak *et al.* (PCR Methods and Applications (1995) 4:357-362) and further in view of the Stratagene Catalog. Applicant traverses this ground of rejection with respect to the newly amended claims for the reasons discussed below.

Claim 16 is directed to a kit comprising pairs of primers and probes for the detection and differentiation of five subgroups of pathogenic *E. coli*. As noted by the Examiner, the Statagene Catalog lists gene characterization kits. Absent a teaching of a kit directed primers and probes for the detection and differentiation of five subgroups of pathogenic *E. coli*, the Startagene Catalog cannot render the claimed invention obvious. As discussed above Lange et al and Livak et al do not remedy this deficiency. Therefore, the Stratagene Catalog cannot render the claimed invention obvious. Withdrawal of this ground of rejection is respectfully requested.

Claims 17 and 18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* in view of Ohashi *et al.* (EP 0566504 A2). Applicant traverses this ground of rejection with respect to the newly amended claims for the reasons discussed below.

Ohashi et al. relates to a PCR method for the detection of the heat-labile and the heat stabile toxin gene of enterotoxigenic *E. coli*. Specifically, Ohashi et al detects one subgroup of pathogenic E. coil by two different characteristic toxin genes and not detection and differentiation of all five subgroups of pathogenic *E. coli*. As discussed above, Lang *et al.* relates to the amplification of a toxin gene characteristic for one of the two subgroups (e.g., enterotoxigenic and enterohemorrhagic *E. coli* strains) and not detection and differentiation of all five subgroups of pathogenic *E. coli*. Thus, at most the combination of Lange and Ohashi would allow the detection of two subgroups and would not provide a definite diagnosis of pathogenic *E. coli*. Such a method is found only in the instant application. accordingly, withdrawal of this ground of rejection is respectfully requested.

Claims 17, 19, and 20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Lang *et al.* in view of Tsen *et al.* (Journal of Food Protection (1996) Vol. 59, No. 8, pp. 795-80).

Applicant traverses this ground of rejection with respect to the newly amended claims for the reasons discussed below.

Like the Ohashi et al application, Tsen et al relates to a PCR method for the detection of the heat-labile and the heat stabile toxin gene of enterotoxigenic *E. coli* (i.e., detects one subgroup of pathogenic *E. coli* by two different characteristic toxin genes) and not detection and differentiation of all five subgroups of pathogenic *E. coli*. As discussed above, Lang *et al.* does not remedy the deficiency of Tsen et al. Accordingly, these references either alone or in combination cannot render the claimed invention obvious. Withdrawal of this ground of rejection is respectfully requested.

CONCLUSION

Applicants respectfully submit that the claims comply with 35 U.S.C. § 112, first and second paragraph and define an invention that is patentable over the art. Accordingly, allowance is in order, and an early notification to that effect would be appreciated. Should the Examiner in reviewing the communication have any questions or need any additional information, she is welcome to contact the undersigned at (650) 849-4902.

Version of the Amendments to the Specification with Markings to Show Changes Made

For the the paragraph on page 5, lines 7-1:

-- TAQMAN™- [TaqMan™-] PCR is performed in optical tubes that allow measurements of fluorescence signals without opening the PCR tubes. This dramatically minimizes post-PCR processing time and almost completely eliminates cross-PCR contamination problems. Employing this approach, simultaneous testing of biological materials for the presence of virulence genes of *E.coli* strains and other enterobacteria, harboring virulence genes can be semiautomated and performed within 18 h. --

For the paragraph on page 5, lines 15-19:

-- According to the present invention Real Time PCR (e.g., TAQMAN™ PCR) [TaqMan™-PCR] for the detection of pathogenic *E.coli* is provided, enabling for the first time the specific, rapid and high throughput routine detection of EHEC, ETEC, EPEC, EIEC, and EaggEC and related enterobacteria that harbor these virulence genes in routine bacteriological laboratories. --

For the the paragraph on page 16, lines3-12:

-- various post-PCR steps in order to verify whether specific amplification of a target gene has occured (68J2). The TAQMAN™-PCR [TaqMan™-PCR] detection system (74,75,90) enables the rapid, specific, sensitive, and high-throughput diagnosis for differentiation of pathogenic Lcoli strains from other strains of *E. coli.* The assay has the ability to quantify the intial target sequence. Since PCR-reaction tubes have not to be opened after PCR cycling, the potential danger of cross-PCR contamination is almost neglegible. The scanning time of 96 samples is approximately 8 min, and calculation of test results can be automated with a commercially available spred sheet program. Thus, overall post-PCR processing time is cut to a minimum.--

For the paragraph on page 16, lines14-30:

-- The TAQMAN™ [TaqMan™]-System relies on standard PCR technique with the addition of a specific internal fluorogenic oligonucleotide probe. The combination of

conventional PCR with the Taq polymerase-dependent degradation of an internally hybridized ohgonucleotide probe confers also specificity to this detection method, since it is highly unlikely that unspecific PCR amplification will yield positive fluorescence signals. Some rules for chosing the fluorigenic probes have to be obeyed (74,75). Criticial are the lenght of the probe, the location of reporter and quencher dyes and the absence of a guanosine at the 5'-end (74). Also, the distance of the probe from one of the specific PCR primers is important. This is due to the fact that the probe has to stay annealed to the template strand in order to be cleaved by Taq polymerase. Since annealing depends, at least partially, on the Tm of the probe, probes should be designed to have a higher Tm as the primers. According to the present invention this was solved (except for sltII) by designing probes that were 3 to 6 bp longer than the specific primers. PCR amplification includes extension of the target sequence after annealing of the primers and the Tm of the extended primers increases. For ...--

For the paragraph on page 17, lines 2-15:

-- the fluorogenic oligonucleotide probe, where the 3'- end is capped in order to avoid elongation, the Tm remains constant, making it more likely that the probe dissociates before degradation by Taq polymerase. Oligonucleotide probe degradation can be optimized by spatial proximity of the fluorogenic probe and the primer. By moving the probe for sltI from 121 bp to 9 bp close to the primer, a significant improvement in ARQ values could be obtained. A second strategy of optimization of TAQMAN™-PCR [TaqMan™-PCR] is to perform PCR elongation at 65^0C, where it is also less likely that the probe dissociates from the template strand before Taq polymerase reaches and hydrolizes it. Values for ΔRQ can thus again be increased about 1.2 to 1.5 fold. The increase of ΔRQ values might be due to the ratio of annealed oligonucleotide probe reached by Taq polymerase or to an increased processivity of Taq polymerase.--

For the paragraph on page 17, lines 17-30:

-- The concentration of fluorogenic probes influences the accuracy of TAQMAN™ [TaqMan™]-results. When the probe concentrations were > 50 pmol / PCR reaction only a relatively small fraction was hydrolysed by Taq polymerase. The ratio of undegraded probe to degraded probe remains high and the fluorescence emmission of the unquenched reporter dye

does not significantly increase in relation to the fluorescence intensity of the reporter dye sill close to the quencher. Thus, at high probe concentrations, ΔRQ values are lower than with intermediate probe concentrations (10 20 pmol). When the probe concentration is too low, ΔRQ values are increased, however, variability of PCR results is increased, since probably small errors in pipeting or minimal differences between PCR reactions become critical. Optimal probe concentration that yielded smallest variabilties and highest RQ values were found at a probe concentration of 20 pmol.--

For the paragraph on page 18, lines 3-15:

-- Since TAQMAN™-PCR [TaqMan™-PCR] uses an internal oligonucleotide probe for detection of template amplification, specific primers and probes can be amply designed. The design of primer and probe sequences is especially important, when nucleotide sequence variants of a given gene exist. This is the case for sltI and sltII. For sltI, all published sequences were aligned and primers and probes were designed to bind to conserved regions of all three variants. For sltII, only one region of the published genes was conserved, thus this region was chosen for the fluorogenic oligonucleotide probe. The primers for amplification of sltII were designed to contain all possible nucleotide sequences at the ambiguous positions of the published sltII variants (degenerate primer approach) (79-83). By employing degenerate primers, it is possible to detect all published variants in one single PCR reaction.--

For the paragraph on page 18, lines 17-26:

-- The isolation method for template DNA affects the performance of the PCR. Two methods, that are suited as rapid purification steps for routine applications, namely boiling prep or spin prep were compared. Boiling preps may still contain some bacterial components that can affect PCR reactions, however, it is extremely fast. The spin prep method involves isolation steps that serve to purify DNA from potentially negatively influencing materials. ΔRQ values and sensitivity of TAQMAN™-PCR [TaqMan™-PCR] for virulence genes from enterobacteria was not found significantly increased as compared to boiling preps when template DNA was prepared by spin prep method.--

For the paragraph on page 17, lines 28-30:

-- The overall sensitivity of TAQMAN™-PCR [TaqMan™-PCR] for all primer/probe combinations was comparable to visual scoring of PCR products by detection with ethidium bromide stained agarose gel electrophoresis. Under optimized...--

For the paragraph on page 20, lines 3-21:

-- flora". In order to address this problem a set of specific primers and fluorogenic probes were developed and optimized for TAQMAN™ [TaqMan™]-based detection of virulence factors harbored by these bacteria (Tables 2 and 3). Arranging patient samples, positive and no-template controls of all 8 tested virulence genes in a standard 96 well microtiter format, a turnaround time from preparation of sample DNA to fluorescence measurement of under 5 hours can be achieved. Thus, the TAQMAN™ [TaqMan™]-based assay for pathogenic *E.coli* provides an ultrarapid means of diagnosis of these bacteria. While being accurate, sensitive and specific, this assay requires minimal post PCR processing time compared to conventional methods. When TAQMAN™-PCR [TaqMan™-PCR] is performed in optical tubes also the danger of cross-contamination of PCR reactions with amplified products is reduced to a minimum. Detection of virulence plasmids harbored by pathogenic enterobacteria might prove the potential of these bacteria to cause disease in the host. It is not clear whether enterobacteria that contain toxin genes or attachment factors do also always express them outside the host. This might be an explanation why ELISA tests for shiga like toxins might be negative in a number of HUS cases where sltI and/or sHII containing EHECs can be detected by nucleic acid based methods.--

For the paragraph on page 20, lines 3-30:

-- The TAQMAN™ [TaqMan]-assay according to the invention for detection of pathogenic Ecoli was then tested in a routine diagnostic setting for the examination of stool samples obtained from children with diarrhea within a defined geographic area (Southern Bavaria) during a 7 month period. Results obtained by TAQMAN™-PCR [TaqMan™-PCR] were compared to the standard detection method for PCR products (electrophoresis of ethidium stained agarose gels). 100 stool samples were analysed (Table 4). 22% of samples were found to test positive for one or more virulence factors. There were 2 cases....--

For the paragraph on page 21, lines14-24:

--Collectively, these investigations show that a large proportion of diarrheal diseases in children and also in adults are associated with pathogenic *E. coli* that are falsely diagnosed as commensal flora in standard microbiological procedures. The TAQMAN™ [TaqMan™] methodology according to the invention for the first time enables the direct, fast, specific, and sensitive detection of these important pathogens. Moreover, virulence genes detected with this approach are not confined to *E. coli,* they also can be freely transmitted to other enterobacteria. Detection of the virulence genes within these bacteria would also be covered by the herein described TAQMAN™-PCR [TaqMan™-PCR]. The assay requires only minimal post-PCR detection time, can thus be performed under 18 hours, and abolishes PCR-cross contamination problems. --

For the paragraph on page 22, lines 27-30:

-- TAQMAN™-PCR [TaqMan™-PCR] was optimized by isolation of DNA from *E. coli* control strains harboring genes for LT, ST, inv-plasmid, pCVD342, EAF, eae, sltI and sltII (see Table 1). $MgCl_2$ concentrations were adjusted for maximum PCR..--

For the paragraph on page 23, lines 24-30, and page 24, lines 3-6:

--The influence of DNA preparation on the performance of TAQMAN™-PCR [TaqMan™-PCR] was tested, since it has been reported that crude bacterial lysates can contain inhibiting factors that might interfere with PCR performance. Therefore, bacteria were collected after overnight growth on McConkey plates. DNA was prepared by boiling of bacteria inoculated in 0.9% NaCl solution or by isolation of genomic DNA with a commercial spin prep procedure (see the example, material and methods). The RQ values and sensititvity of TAQMAN™-PCR [TaqMan™-PCR] did not differ when the two preparation methods were compared. The RQ values obtained for PCR amplifications from DNA derived from 10^5 SltI or sltII containing EHEC prepared by boiling or by spin prep comparable. --

For the paragraph on page 24, lines 8-15:

--The TAQMAN™-PCR [TaqMan™-PCR] method relies on the detection of free reporter dye (FAM) that is released from the probe after hydrolysis. Thus, probe concentration

should also have an effect on the assay performance by affecting the fraction of the probe that is degraded during PCR cycling. Probe concentrations were titrated in the range of 100 pmol to 0.1 pmol and WQ values were determined. Optimal probe concentrations varied in between 10 pmol and 20 pmol depending on the target gene that was amplified. --

For the paragraph on page 24, lines 17-30, and page 25, lines 3-4:

-- For testing sensitivity of TAQMAN™-PCR [TaqMan™-PCR], EHEC containing either sltl or sltll were diluted in a suspension containing E.coli strain ATCC11775 at 10^7 CfU at log step dilutions. PCR was performed under optimized conditions and results from ethidium-bromide stained agarose gels were compared to TAQMAN™ [TaqMan™] results. Minimum detection limits of a sltl containing EHEC strain was 10^3 cfu within 10^7. For sltll the detection limit was found at $10^{3.5}$ cfu in 10^7 enterobacteria. Both methods, detection of PCR products by agarose gel electrophoresis and measurement of fluorescence signals by the TaqMan method yielded comparable results, i.e. that at ΔRQ values above ΔRQ threshold PCR product bands were visible in agarose gelb whereas at ΔRQ values around ΔRQ threshold also in agarose gels PCR products were below the detection limit. After optimizing detection tests for all virulence factors/ toxins, TAQMAN™-PCR [TaqMan™-PCR] was set up for routine testing of biological specimen for the presence of pathogenic E.coli bacteria. Results of TAQMAN™-PCR [TaqMan™-PCR] were compared to agarose gel electrophoresis. . –

For the paragraph on page 25, lines 15-19:

-- In order to verify TAQMAN™-PCR [TaqMan™-PCR] performance and to test for the occurence of pathogenic E.coli screening of 100 stool specimens from children of age 0 to 10 years with the clinical symptoms of diarrhea was undertaken. The materials and methods used in the test are described in more detail below under item 2.--

For the paragraph on page 26, lines 17-23:

-- Enterobacteria from the two patients with EHEC were hybridized with sltl and sltII gene probes for testing accuracy and specificity of TAQMAN™-PCR [TaqMan™-PCR]. In the case of patient one, where TAQMAN™-PCR [TaqMan™-PCR] was positive for sltl, only

colonies hybridizing with sltI could be found. Colonies of patient two, where TAQMAN™-PCR [TaqMan™-PCR] was positive for sltI and sltII, hybridized with probes for sltI and sltII. Positive colonies were picked and biochemically typed as *E. coli*--.

For the paragraph on page 29, lines 3-11:

-- e) Sensitivity of TAQMAN™ [TaqMan™] technique: For determination of the sensitivity of the TAQMAN™ [TaqMan™] method, serial log-step dilutions of positive control strains were performed in a solution containing 107 cfu of Ecoli reference strain ATCC 11775 DNA was either prepared by the boiling method (see above) or purified using spin prep columns designed for isolation of genomic bacterial DNA (Qiagen, Germany). Purification was according to the protocol of the manufacturer. The detection limit for sltI containing strains was determined with 10^3 cfu among 10^7 E.coli and for sltII containing strains as $10^{3.5}$ among 10^7.

For the paragraph on page 29, lines 13-31:

-- f) Colony hybridisation and isolation of EHEC bacteria: EHEC bacterial strains and stool samples from patients testing positive in sltI or sltII TAQMAN™-PCR [TaqMan™-PCR] were subjected to colony hybridisation. Briefly, bacteria were plated on McConkey agar plates such that single colonies could be seen. Bacteria were blotted on nylon membranes (Genescreen Plus, NEN, Germany), cracked (1% SDS), denatured (0.5M NaOH, 1.5M NaCl), neutralized (1M TRIS, 1.5M NaCl), and washed (20xSSC). Membranes were baked at 80^0C for 2 hours. DNA probes specific for sal or sltII were labelled with fluorescein (Gene-Images random prime labelling module, Amersham, Germany). Afterwards, filters were hybridized with labelled probes. Hybridization was verified by non-radioactive detection system employing anti-FITC peroxidase mAb and ECL detection module (Gene Images CDP-Star detection module, Amersham, Germany). Bacterial colonies hybridizing with the probe and non-hybridizing colonies were picked, verified by TAQMAN™-PCR [TaqMan™-PCR] and tested for antibiotic susceptibility. Antibiotic susceptibility testing. EHEC and non-EHEC Ecoli were picked from McConkey plates after testing for sltI or sltII or both toxin genes in colony hybridazation.and MIC testing was performed according to NCCLS guidelines for enterobacteria.

Version of the Amendments to the Claims with Markings to Show Changes Made

1. (Amended). A Polymerase Chain Reaction (PCR) method for detection and differentiation of pathogenic enterobacteria in a sample, wherein a set of oligonucleotide primer pairs is added to said sample, each primer pair being capable of specifically amplifying a DNA sequence of a virulence factor/toxin gene characteristic for one of the subgroups of pathogenic *E. coli*, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one primer is added to the sample [comprising PCR amplification of DNA isolated from said sample using a set of oligonucleotide primer pairs allowing differentiation of at least two groups of pathogenic *E. coli* strains by amplification of a virulence factor/toxin gene characteristic for the respective group of the pathogenic *E. coli* strains].

2. (Amended) The method according to claim 1 wherein the set of oligonucleotide primer pairs comprises primer pairs [two or more primer pairs] selected from

a primer pair that hybridizes to a gene encoding heat labile toxin, or heat stabile toxin for amplification of a DNA sequence characteristic for enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stabile toxin for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid for amplification of a DNA sequence characteristic for enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid for amplification a DNA sequence contained in enteroinvasive *E. coli*;

a primer pair that hybridizes to the EAF plasmid, or the eae gene for amplification of a DNA sequence characteristic for enteropathogenic *E. coli*;

a primer pair that hybridizes to the genes encoding shiga-like toxin sltI or sltII for amplification of a DNA sequence characteristic for enterohemorrhagic *E. coli*.

3. (Amended) The method according to claim 2 wherein

the primer pair that hybridises to the gene encoding heat labile toxin characteristic for enterotoxigenic *E. coli* is

LT-I: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO.: 1) and
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO.: 2);

the primer pair that hybridises to the gene encoding heat stabile toxin characteristic for enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO.: 3) and
ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO.: 4);

the primer pair that hybridises for the gene encoding heat stabile toxin characteristic for enteroaggregative *E. coli* is

EASTI-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO.: 5) and
EASTI-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO.: 6) ;

the primer pair which hybridises to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO.: 7) and
EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO.: 8);

the primer pair which hybridises to the inv-plasmid is

EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO.: 9) and
EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO.: 10);

the primer pair which hybridises to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO.: 11) and
EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO.: 12);

the primer pair which hybridises to the eae gene is

EPeh-1: 5' CCC GCA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO.: 13) and
EPeh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO.: 14);

the primer pair which hybridises to the gene encoding shiga-like toxin SltI is

SltI-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO.: 15) and
SltI-2 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO.: 16);

the primer pair which hybridises to the gene encoding shiga-like toxin SltII is

SltII-1: 5' ATG AAG AAG ATR WTT RTD GCR CYT TTA TTY G 3' (SEQ ID NO.: 17) and

SltII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3' (SEQ ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

4. (Amended). The method according to claim 1 wherein a polymerase having additional 5'-3' exonuclease activity is used for the amplification of DNA, and an oligonucleotide probe labeled at the most 5' base with a fluorescent dye and at the most 3' base with a fluorescent quencher dye [which] and wherein said probe hybridizes within the target DNA is included in the amplification process; said labeled oligonucleotide probe being susceptible to 5'-3' exonuclease degradation by said pol.

7. (Amended) The method according to claim 6 wherein the labelled oligonucleotide probe for the detection of heat labile toxin characteristic for enterotoxigenic *E. coli* is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG 3' (SEQ ID NO.: 19)

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enterotoxigenic *E. coli* is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3' (SEQ ID NO.: 20);

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enteroaggregative *E. coli* is

5' ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3' (SEQ ID NO.: 21);

the labelled oligonucleotide probe for the detection of pCVD432 plasmid is

3' CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3' (SEQ ID NO.: 22);

the labelled oligonucleotide probe for the detection of the inv-plasmid is

5' CAA AAA CAG AAG AAC CTA TGT CTA CCT 3' (SEQ ID NO.: 23)

the labelled oligonucleotide probe for the detection of the EAF-plasmid is

5' CTT GGA GTG ATC GAA CGG GAT CCA AAT 3' (SEQ ID NO.: 24);

the labelled oligonucleotide probe for the detection of the eae gene is

5' TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3' (SEQ ID NO.: 25);

the labelled oligonucleotide probe for the detection of shiga-like toxin SltI gene is

5' TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3' (SEQ ID NO.: 26);

the labelled oligonucleotide probe for the detection of shiga-like toxin SltII gene is

5' CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3' (SEQ ID NO.: 27).

10. (Amended) A set of oligonucleotide primer pairs useful for polymerase chain reaction (PCR) amplification of DNA of pathogenic enterobacteria allowing detection and differentiation of [at least two different groups of pathogenic *E. coli* strains by amplification] pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for [the respective group of] one of the subgroups of the pathogenic *E. coli* strains, said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one primer is included in said set of oligonucleotide probes.

11. (Amended) The set of primer pairs according to claim 10 comprising [two or more primer pairs selected from]

a primer pair that hybridizes to a gene encoding heat labile toxin, or heat stabile toxin of enterotoxigenic *E. coli*;

a primer pair that hybridizes to a gene encoding heat stabile toxin of enteroaggregative *E. coli*;

a primer pair that hybridizes to the pCVD432 plasmid of enteroaggregative *E. coli*;

a primer pair that hybridizes to the inv-plasmid of enteroinvasive *E. coli*;

a primer pair that hybridizes to the EAF plasmid, or the eae gene of enteropathogenic *E. coli*;

a primer pair that hybridizes to the gene encoding shiga-like toxin sltI or sltII of enterohemorrhagic *E. coli*.

12. (Amended) The set of primer pairs according to claim 11 wherein

the primer pair which hybridises to the gene encoding heat labile toxin of enterotoxigenic *E. coli* is

LT-1: 5' GCG TTA CTA TCC TCT CTA TGT G 3' (SEQ ID NO.: 1) and
LT-2: 5' AGT TTT CCA TAC TGA TTG CCG C 3' (SEQ ID NO.: 2);

the primer pair which hybridises to the gene encoding heat stabile toxin of enterotoxigenic *E. coli* is

ST-1: 5' TCC CTC AGG ATG CTA AAC CAG 3' (SEQ ID NO.: 3) and
ST-2a: 5' TCG ATT TAT TCA ACA AAG CAA C 3' (SEQ ID NO.: 4);

the primer pair which hybridises to the gene encoding heat stabile toxin of enteroaggregative *E. coli* is

EASTI-1: 5' AAC TGC TGG GTA TGT GGC TGG 3' (SEQ ID NO.: 5) and
EASTI-2: 5' TGC TGA CCT GCC TCT TCC ATG 3' (SEQ ID NO.: 6);

the primer pair which hybridises to the pCVD432 plasmid is

EA-1: 5' CTG GCG AAA GAC TGT ATC ATT G 3' (SEQ ID NO.: 7) and
EA-2: 5' TAA TGT ATA GAA ATC CGC TGT T 3' (SEQ ID NO.: 8);

the primer pair which hybridises to the inv-plasmid is

EI-1: 5' TTT CTG GAT GGT ATG GTG AGG 3' (SEQ ID NO.: 9) and
EI-2: 5' CTT GAA CAT AAG GAA ATA AAC 3' (SEQ ID NO.: 10)

the primer pair which hybridises to the EAF plasmid is

EP-1: 5' CAG GGT AAA AGA AAG ATG ATA AG 3' (SEQ ID NO.: 11)
and
EP-2: 5' AAT ATG GGG ACC ATG TAT TAT C 3' (SEQ ID NO.: 12);

the primer pair which hybridises to the eae gene is

EPeh-1: 5' CCC GGA CCC GGC ACA AGC ATA AG 3' (SEQ ID NO.: 13)
and
EPeh-2: 5' AGT CTC GCC AGT ATT CGC CAC C 3' (SEQ ID NO.: 14);

the primer pair which hybridises to the shiga-like toxin sltI gene is

SltI-1: 5' ATG AAA AAA ACA TTA TTA ATA GC 3' (SEQ ID NO.: 15) and
SltI-2: 5' TCA CYG AGC TAT TCT GAG TCA AGC 3' (SEQ ID NO.: 16);

the primer pair which hybridises to the shiga-like toxin sltII is

SltII-1: 5' ATG AAG AAG ATR WTT RTD GCR GYT TTA TTY G 3' (SEQ ID NO.: 17) and

SltII-2: 5' TCA GTC ATW ATT AAA CTK CAC YTS RGC AAA KCC 3' (SEQ ID NO.: 18)

wherein W is A/T, R is A/G, D is A/G/T, Y is C/T and K is G/T.

13. (Amended) A set of labeled oligonucleotide probes useful for detection and differentiation of pathogenic enterobacteria [by TaqMan™-PCR being specific for] in a sample by Real Time–PCR, each probe specifically binding a sequence of a virulence factor/toxin genes characteristic of one of the subgroups of pathogenic *E. coli* strains comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for detection and differentiation of each subgroup at least one probe is included in set of oligonucleotide probes.

15. (Amended) The set of probes according to claim 14 wherein

the labelled oligonucleotide probe for the detection of heat labile toxin characteristic for enterotoxigenic *E. coli* is

5' AGC TCC CCA GTC TAT TAC AGA ACT ATG 3' (SEQ ID NO.: 19);

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enterotoxigenic *E. coli* is

5' ACA TAC GTT ACA GAC ATA ATC AGA ATC AG 3' (SEQ ID NO.: 20);

the labelled oligonucleotide probe for the detection of heat stabile toxin characteristic for enteroaggregative *E. coli* is

5' ATG AAG GGG CGA AGT TCT GGC TCA ATG TGC 3' (SEQ ID NO.: 21);

the labelled oligonucleotide probe for the detection of pCVD432 plasmid is

5' CTC TTT TAA CTT ATG ATA TGT AAT GTC TGG 3' (SEQ ID NO.: 22);

the labelled oligonucleotide probe for the detection of the inv-plasmid is

5' CAA AAA CAG AAG AAC CTA TGT CTA CCT 3' (SEQ ID NO.: 23)

the labelled oligonucleotide probe for the detection of the EAF-plasmid is

5' CTT GGA GTG ATC GAA CGG GAT CCA AAT 3' (SEQ ID NO.: 24)

the labelled oligonucleotide probe for the detection of the eae gene is

5' TAA ACG GGT ATT ATC AAC AGA AAA ATC C 3' (SEQ ID NO.: 25)

the labelled oligonucleotide probe for the detection of shiga-like toxin SltI gene is

5' TCG CTG AAT CCC CCT CCA TTA TGA CAG GCA 3' (SEQ ID NO.: 26);

the labelled oligonucleotide probe for the detection of shiga-like toxin SltII gene is

5' CAG GTA CTG GAT TTG ATT GTG ACA GTC ATT 3' (SEQ ID NO.: 27).

16. (Amended) A kit useful for diagnosing an enterobacteria infection in samples derived from a living animal body including a human, by [TaqMan™-] real time PCR method, said kit comprising:

(a) a set of oligonucleotide primer pairs, wherein said primer pair allows detection and differentiation of [at least two different groups of pathogenic *E. coli* strains by amplification] pathogenic enterobacteria in a sample, wherein each primer pair specifically amplifies a DNA sequence of a virulence factor/toxin gene characteristic for [the respective group of] one of the subgroups of the pathogenic *E. coli* strains said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains and wherein for amplification of each subgroup at least one primer is included in said set of oligonucleotide probes and (b) a set of oligonucleotide probes, wherein said set of oligonucleotide probes detect virulence factor/toxin genes characteristic of one of the subgroups of pathogenic *E. coli* strains , said subgroups comprising enterotoxigenic, enteroaggregative, enteroinvasive, enteropathogenic and enterohemorrhagic *E. coli* strains by real time PCR [TaqMan™-PCR].

The Assistant Commissioner is hereby authorized to charge any additional fees which may be required by this paper, or credit any overpayment to Deposit Account No. 50-1189. Docket No.: 20239-706. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

Dated: June 6, 2001

By: _____

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Respectfully submitted,

Dated: June 6, 2001

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